

Primer Extension Coupled with Electrospray Product Ion Mass Spectrometry

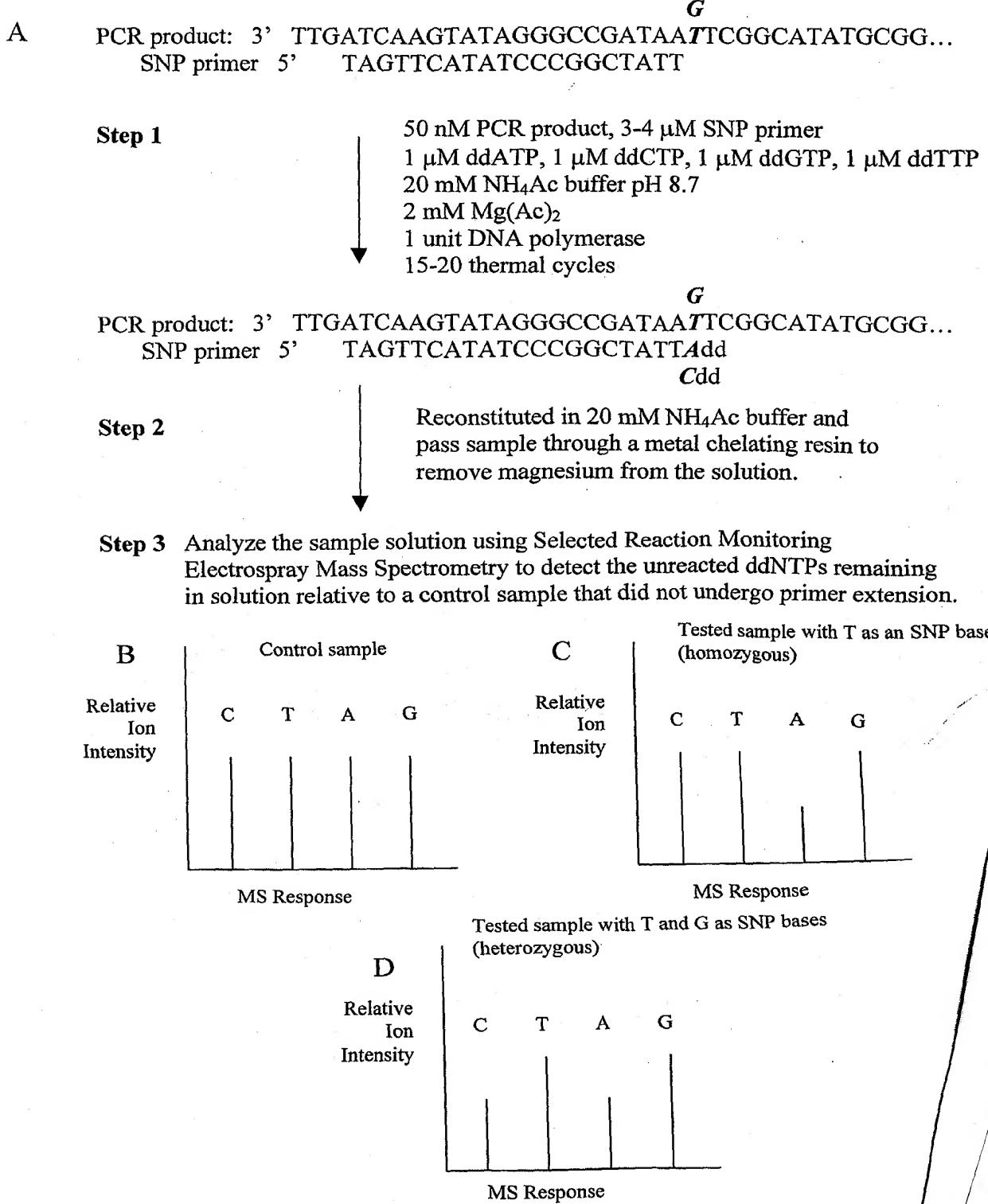


Figure 1

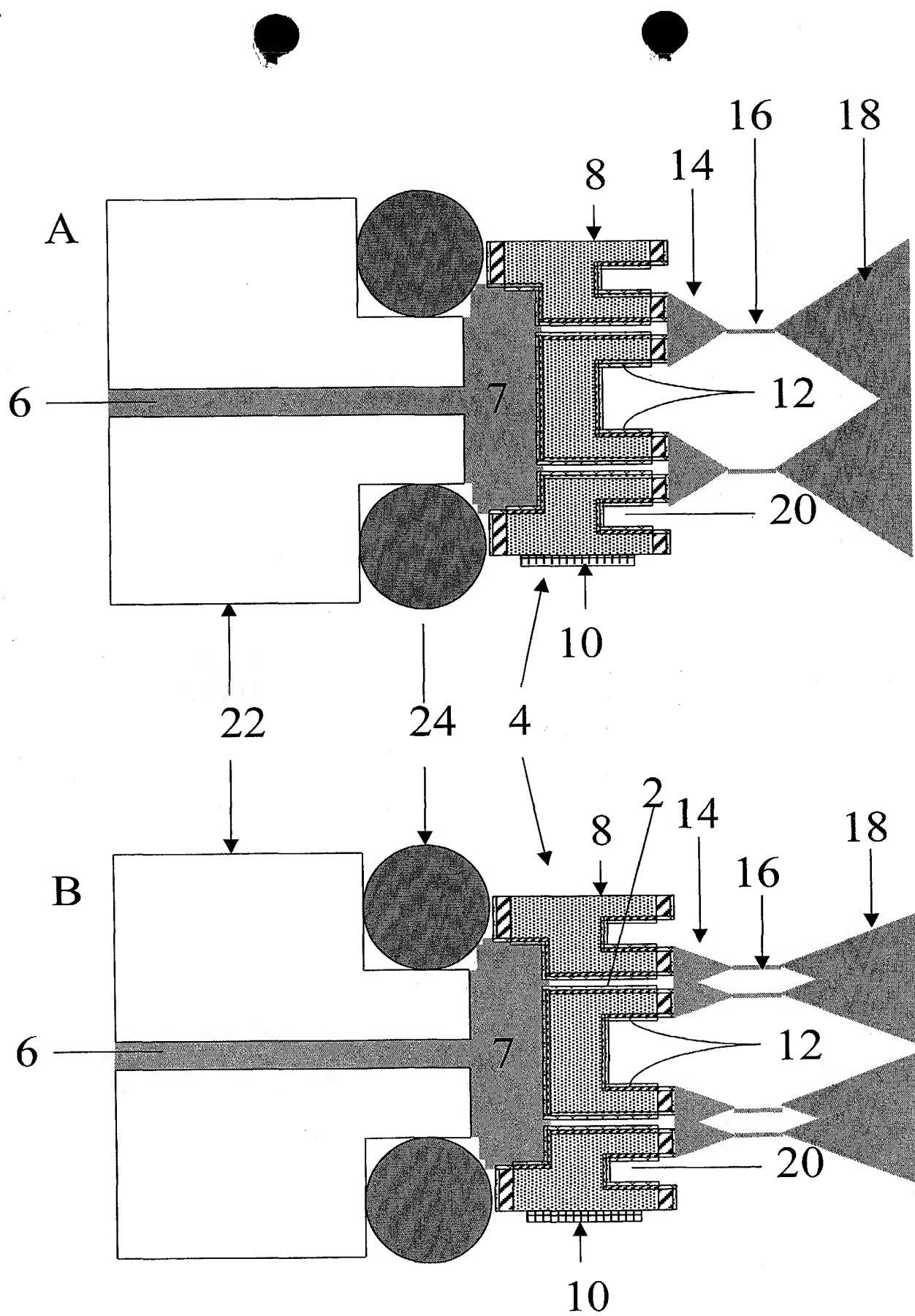


Figure 2

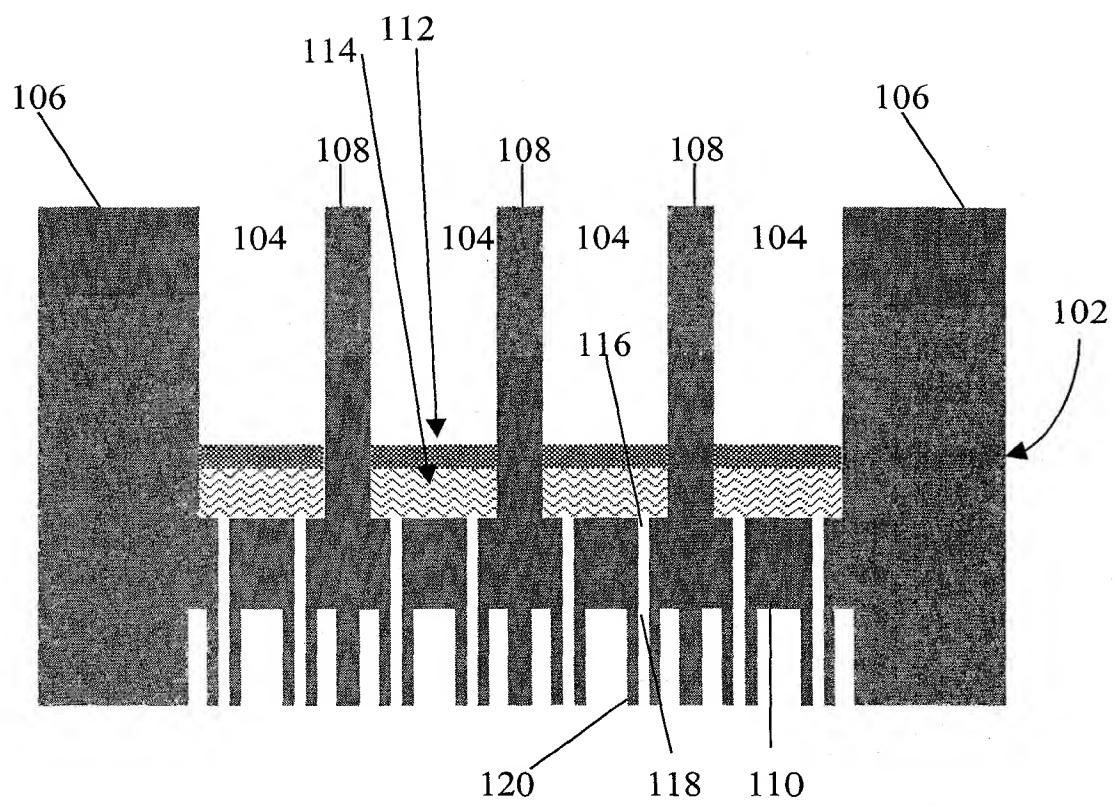


Figure 3A

Pressure to effect fluid transfer

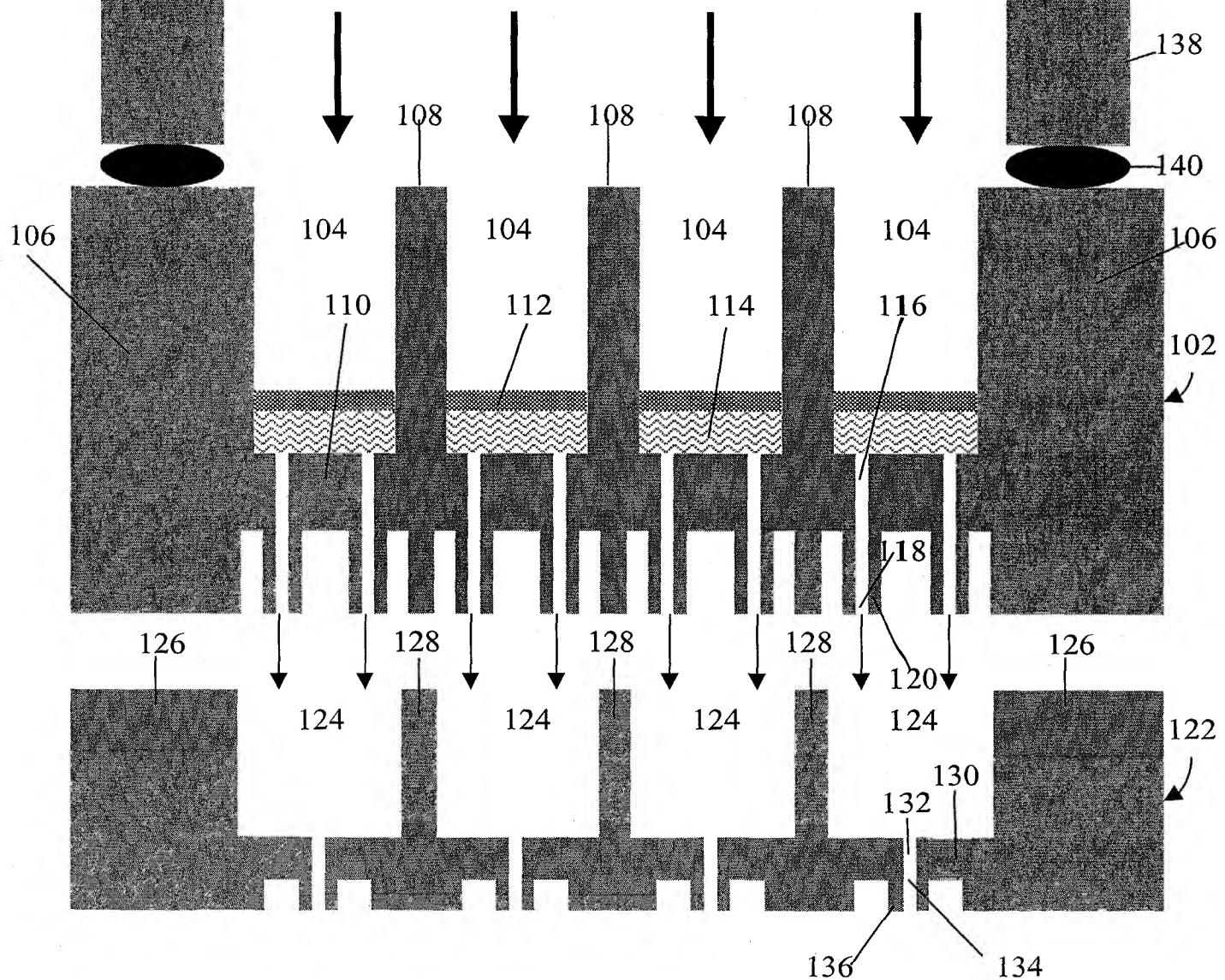


Figure 3B

Fluid
delivery
probe

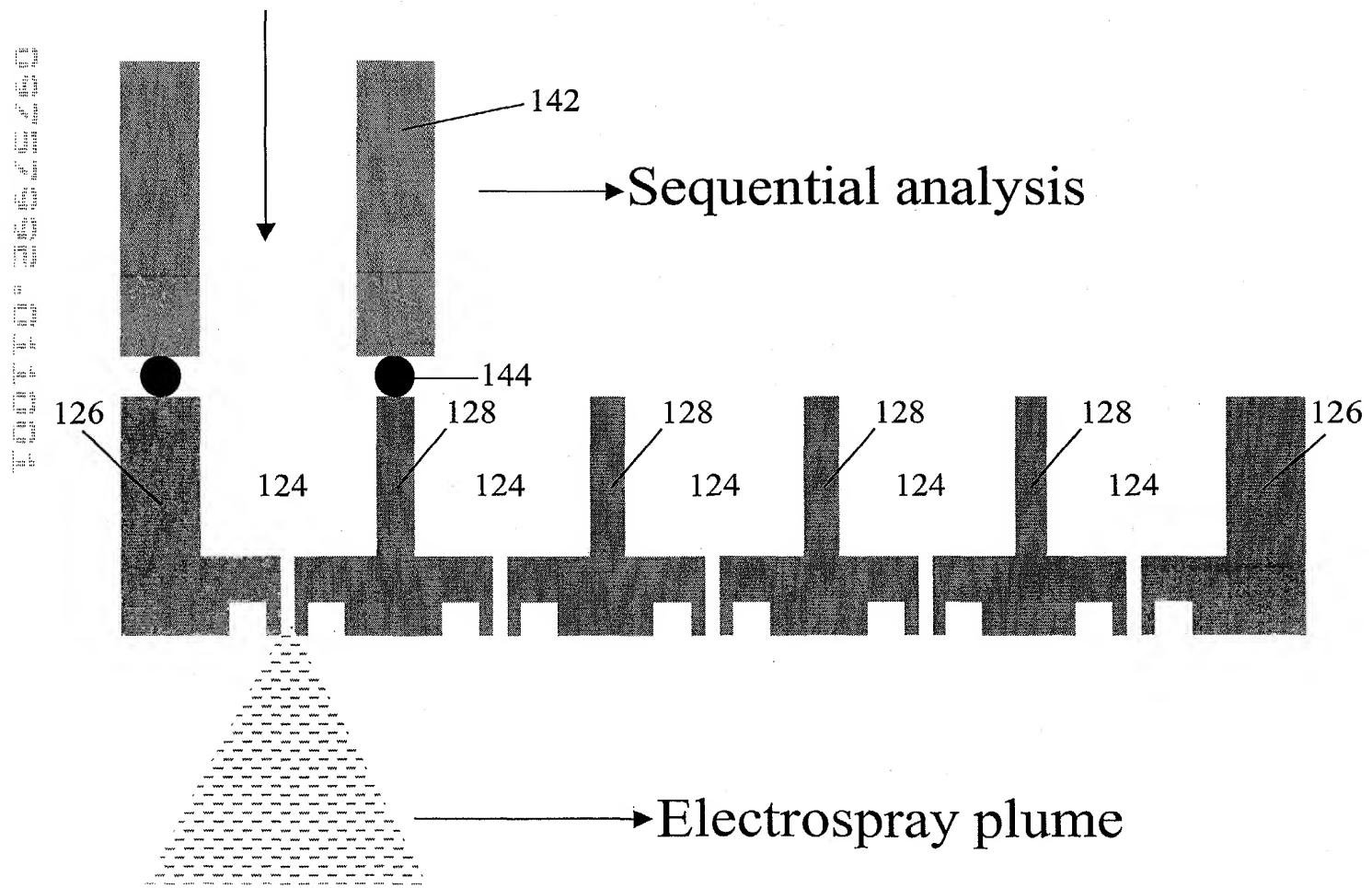


Figure 3C

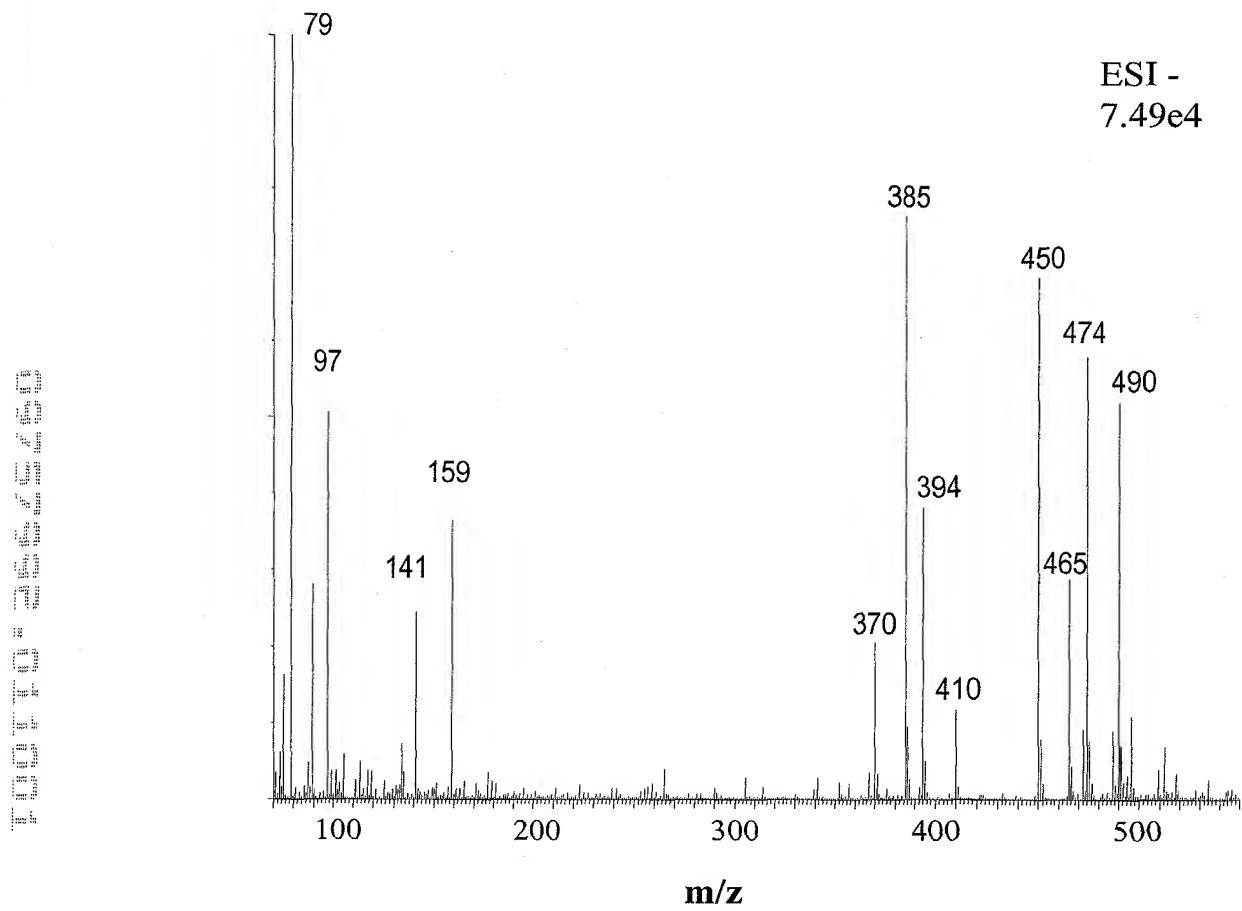


Figure 4

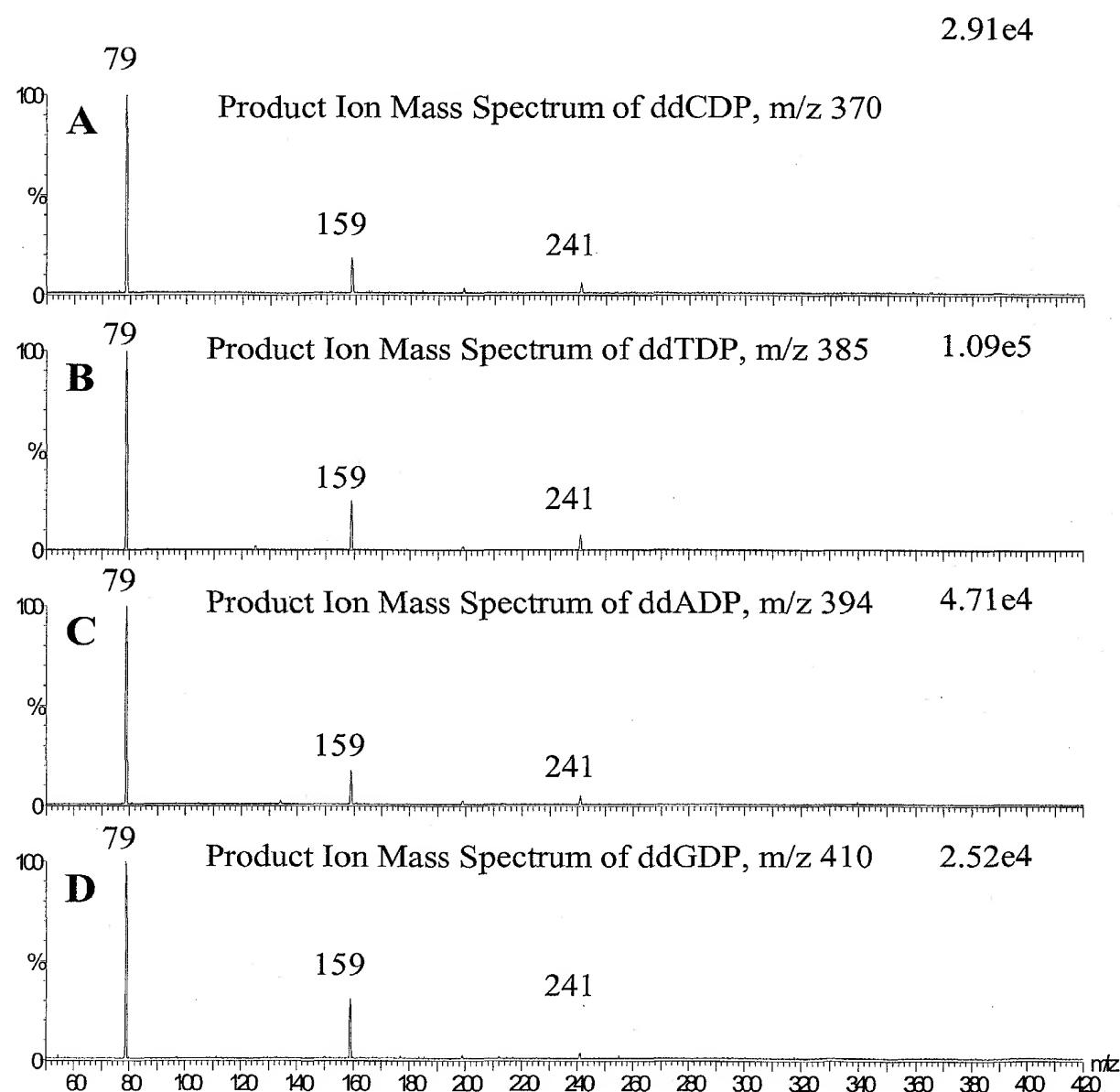


Figure 5

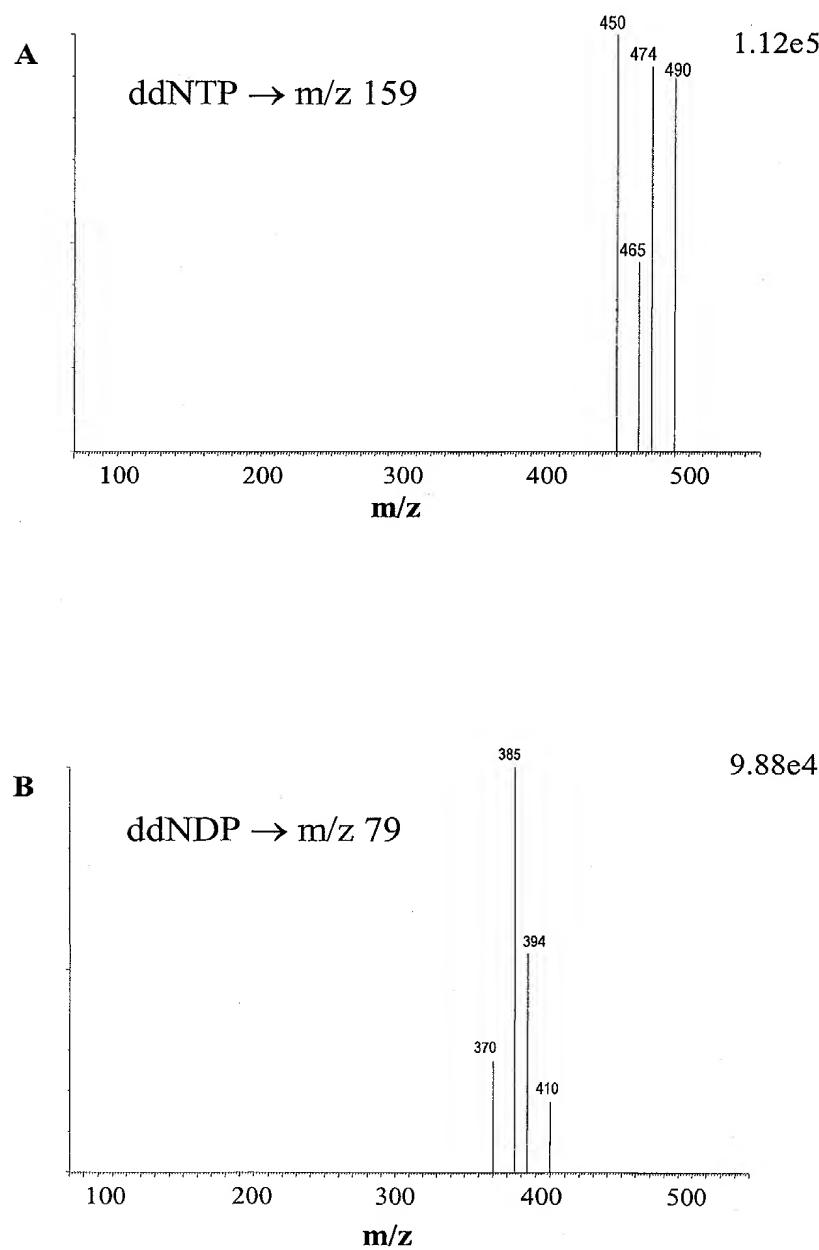


Figure 6

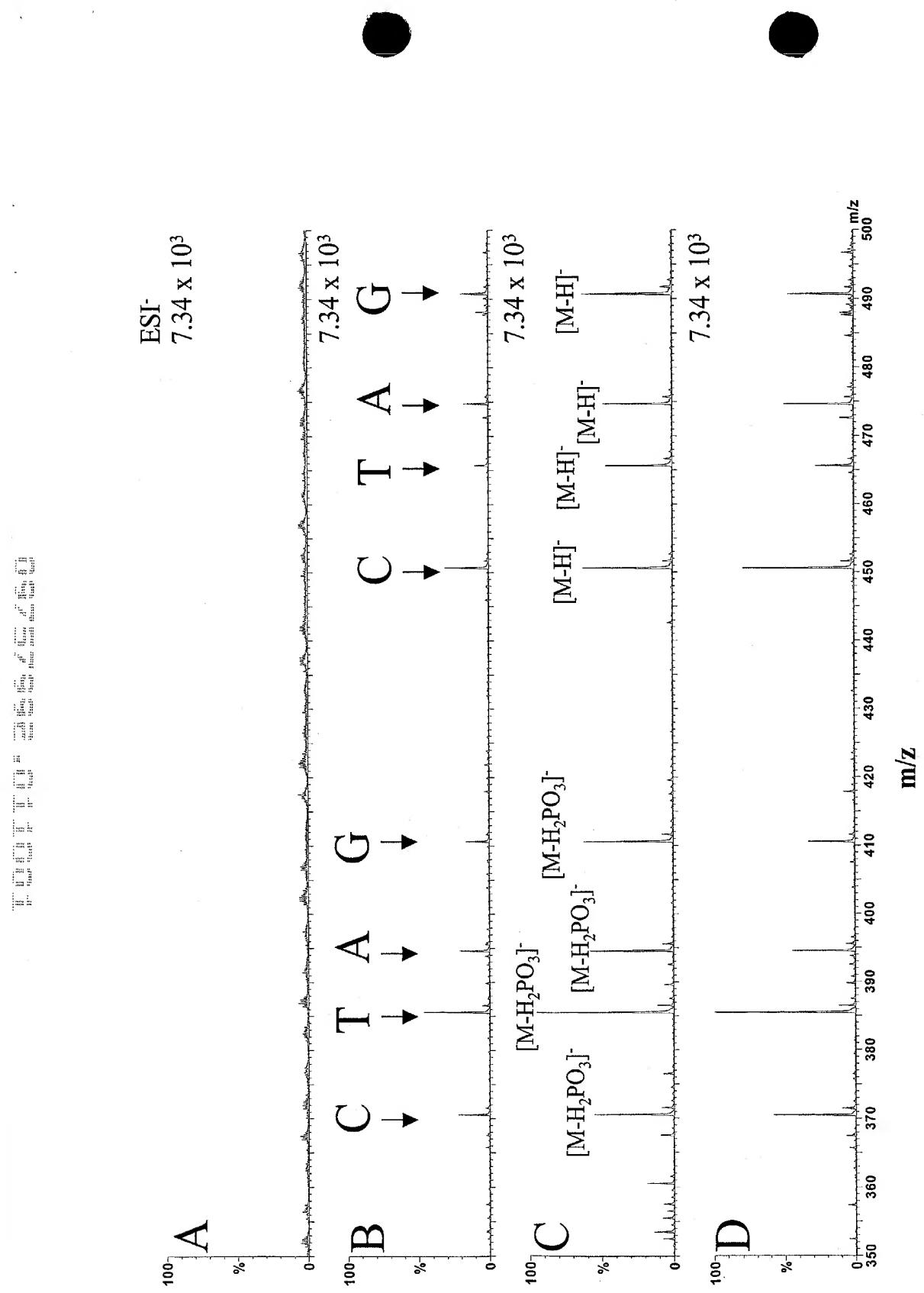


Figure 7

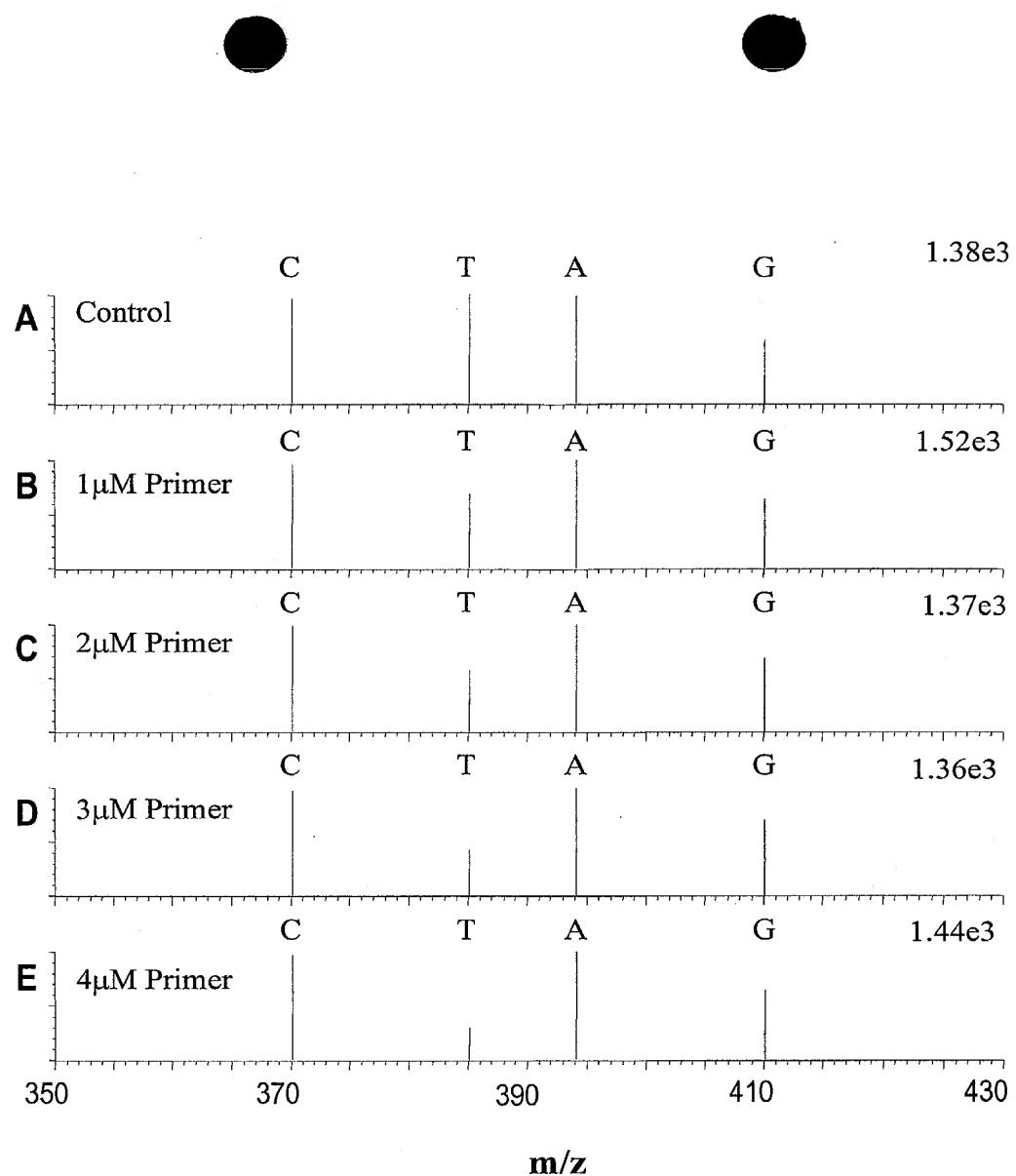


Figure 8

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in homogeneous reaction

Homogeneous templates:

Template A : 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)
SNP primer: 3' AGGACACACACTTTAACAAATAGGCGA 5' (SEQ. ID. No. 5)
Decreased ddNTPs: ddTTP (385.1>79)

Template C : 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)
SNP primer: 3' AGGACACACACTTTAACAAATAGGCGA 5' (SEQ. ID. No. 5)
Decreased ddNTPs: ddGTP (410.1>79)

Template G : 5' CCCCTGT**G**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 3)
SNP primer: 3' AGGACACACACTTTAACAAATAGGCGA 5' (SEQ. ID. No. 5)
Decreased ddNTPs: ddCTP (370.1>79)

Template T : 5' CCCCTGT**T**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 4)
SNP primer: 3' AGGACACACACTTTAACAAATAGGCGA 5' (SEQ. ID. No. 5)
Decreased ddNTPs: ddATP (394.1>79)

Figure 9

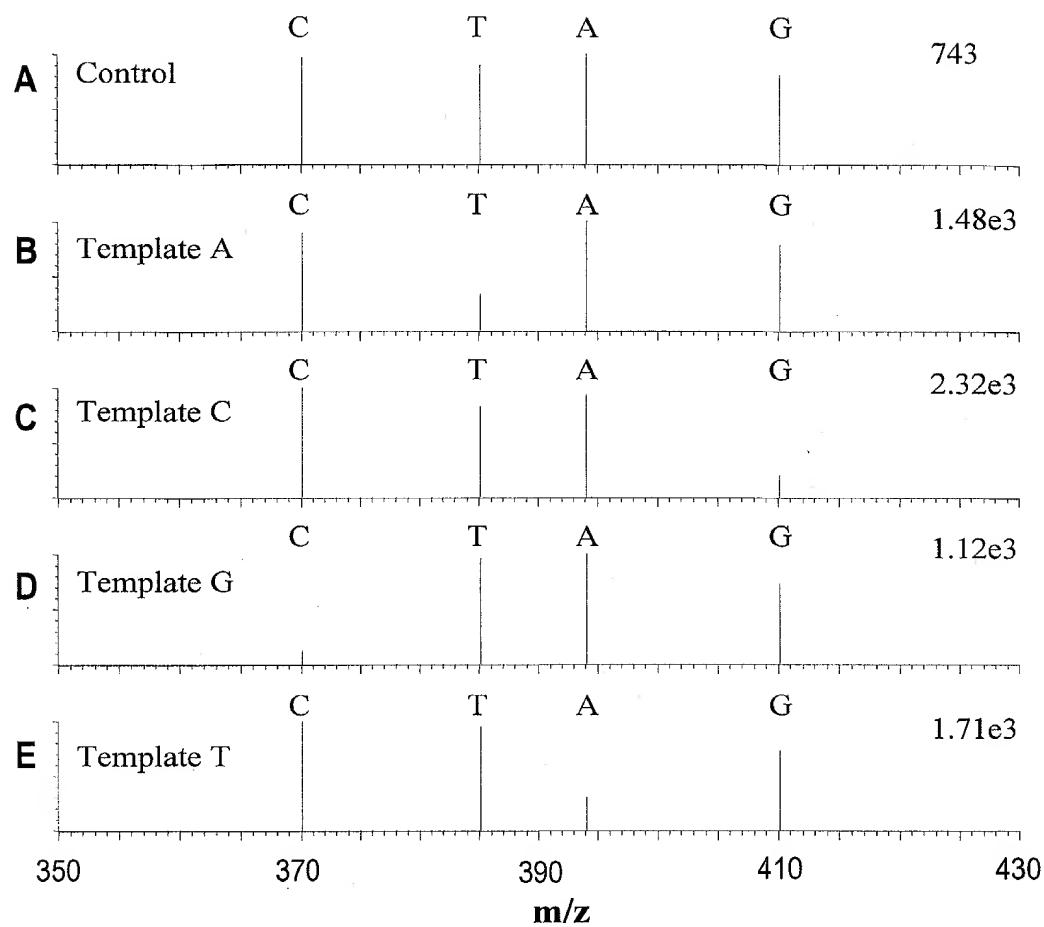


Figure 10

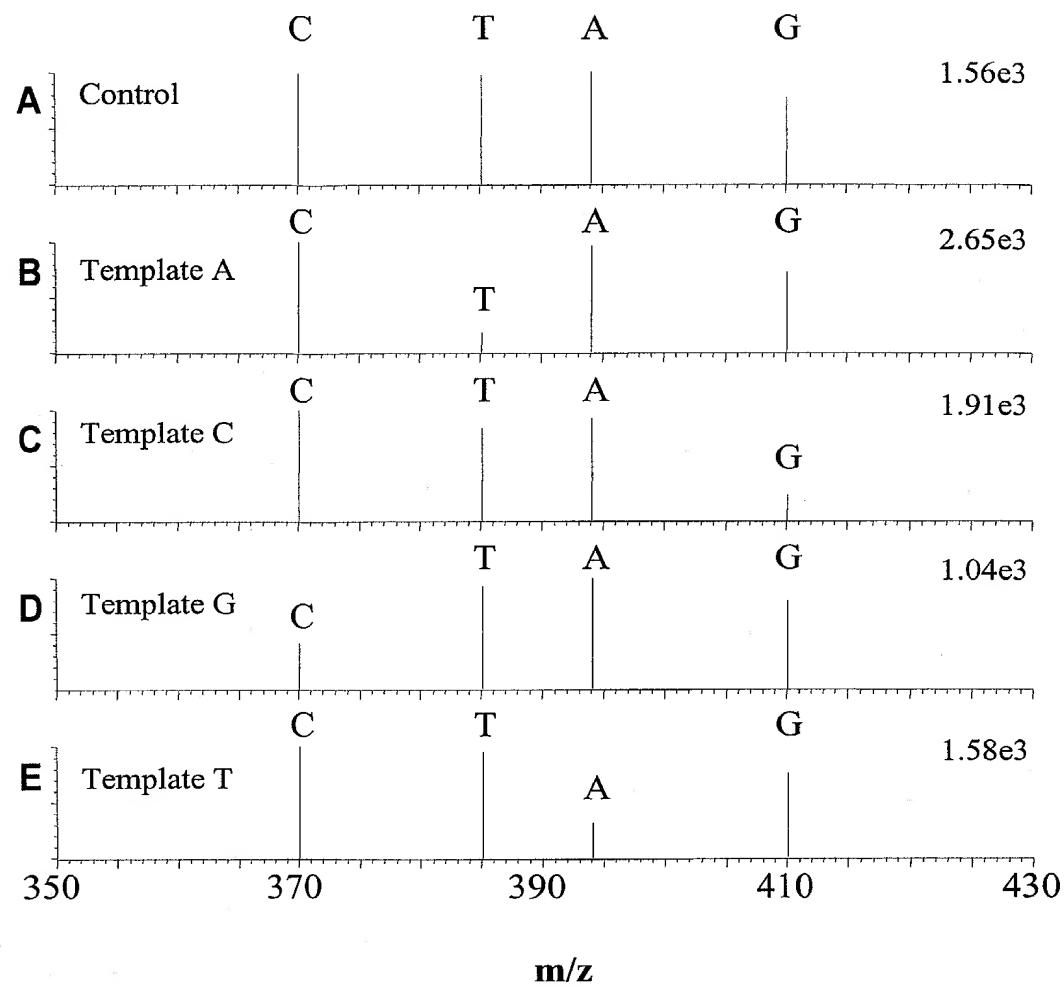


Figure 11

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in heterogeneous reaction

Heterogeneous templates (two templates mixture):

C

Template A+C : 5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC 3'
 SNP primer: 3' AGGACACACTTTAACAAATAGGCGA 5'
 Decreased ddNTPs: ddTTP (385.1>79) and ddGTP (410.1>79)

(SEQ. ID. No. 2)

(SEQ. ID. No. 1)

(SEQ. ID. No. 5)

G

Template A+G : 5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC 3'
 SNP primer: 3' AGGACACACTTTAACAAATAGGCGA 5'
 Decreased ddNTPs: ddTTP (385.1>79) and ddCTP (370.1>79)

(SEQ. ID. No. 3)

(SEQ. ID. No. 1)

(SEQ. ID. No. 5)

T

Template A+T: 5' CCCCTGTATCCTGTGTGAAATTGTTATCCGCTC 3'
 SNP primer: 3' AGGACACACTTTAACAAATAGGCGA 5'
 Decreased ddNTPs: ddTTP (385.1>79) and ddATP (394.1>79)

(SEQ. ID. No. 4)

(SEQ. ID. No. 1)

(SEQ. ID. No. 5)

G

Template C+G: 5' CCCCTGTCTCCTGTGTGAAATTGTTATCCGCTC 3'
 SNP primer: 3' AGGACACACTTTAACAAATAGGCGA 5'
 Decreased ddNTPs: ddGTP (410.1>79) and ddCTP (370.1>79)

(SEQ. ID. No. 3)

(SEQ. ID. No. 2)

(SEQ. ID. No. 5)

T

Template C+T: 5' CCCCTGTCTCCTGTGTGAAATTGTTATCCGCTC 3'
 SNP primer: 3' AGGACACACTTTAACAAATAGGCGA 5'
 Decreased ddNTPs: ddGTP (410.1>79) and ddATP (394.1>79)

(SEQ. ID. No. 4)

(SEQ. ID. No. 2)

(SEQ. ID. No. 5)

T

Template G+T: 5' CCCCTGTGTCCTGTGTGAAATTGTTATCCGCTC 3'
 SNP primer: 3' AGGACACACTTTAACAAATAGGCGA 5'
 Decreased ddNTPs: ddCTP (370.1>79) and ddATP (394.1>79)

(SEQ. ID. No. 4)

(SEQ. ID. No. 3)

(SEQ. ID. No. 5)

Figure 12

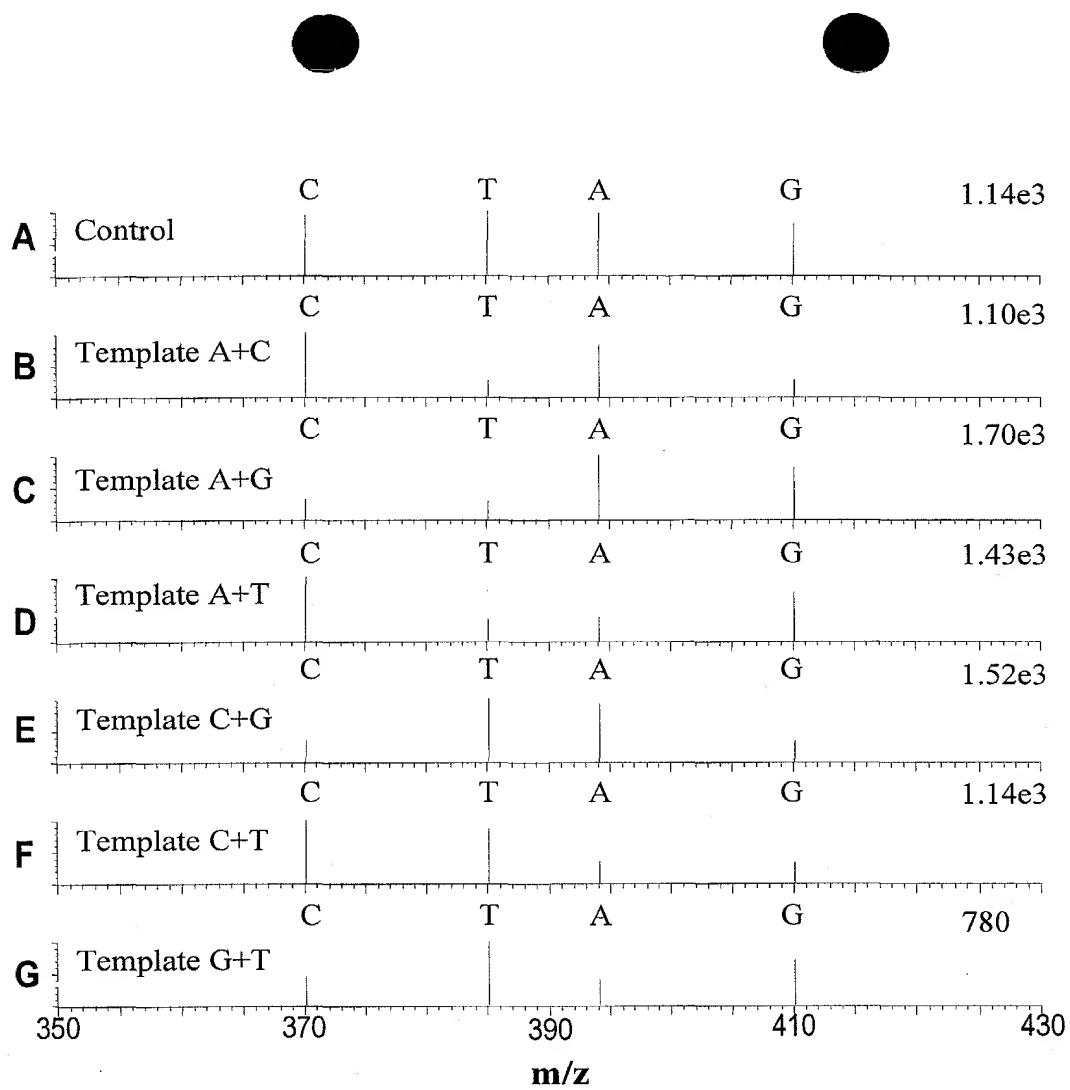


Figure 13

384 bp of partial PheA gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 6)

5' CGGTAATCCA TGGGAAGAGA TGTTCTATCT GGATATTCAAG GCCAATCTTG AATCAGCGGA
3' GCCATTAGGT ACCCTTCTCT ACAAGATAGA CCTATAAGTC CGGTTAGAAC TTAGTCGCCT

5' AATGCAAAAA GCATTGAAAG AGTTAGGGGA AATCACCCGT TCAATGAAGG TATTGGGCTG
3' TTACGTTTT CGTAACCTTC TCAATCCCCT TTAGTGGCA AGTACTTCC ATAACCCGAC

5' TTACCCAAGT GAGAACGTAG TGCCTGTTGA TCCAACCTGA TGAAAAGGTG CCGGATGATG
3' AATGGGTTCA CTCTTGCATC ACGGACAACT AGGTTGGACT ACTTTCCAC GGCCTACTAC

5' TGAATCATCC GGCACTGGAT TATTACTGGC GATTGTCATT CGCCTGACGC AATAACACGC
3' ACTTAGTAGG CCGTGACCTA ATAATGACCG CTAACAGTAA GCGGACTGCG TTATTGTGCG

5' GGCTTCACT CTGAAAACGC TGTGCGTAAT CGCCGAACCA GAATTGAGC TCGGTACCCG
3' CCGAAAGTGA GACTTTGCG ACACGCATTA GCGGCTTGGT CTTAAGCTCG AGCCATGGC

5' GGGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTACAAC
3' CCCTAGGAGA TCTCAGCTGG ACGTCCGTAC GTTCGAACCG TGACCGGCAG CAAAATGTTG

5' GTCGTGACTG GGAAAACCTT GGCG 3'
3' CAGCACTGAC CCTTTGGGA CCGC 5'

5' #1224

Amplification primers:

W338Ip 5'-CGGTAATCCAATTGAAGAGAGATGTTCT-3' (SEQ. ID. No. 7)

#1224 5'-CGCCAGGGTTTCCCAGTCACGA-3' (SEQ. ID. No. 8)

Polymorphism detection primers:

	Polymorphic base	
W338Ip 5'-CGGTAATCCAATTGAAGAGAGATGTTCT-3'	T	(SEQ. ID. No. 7)
C374Spu 5'-TCACTTGGTAGGATCCCAATACCTTCATT-3'	C	(SEQ. ID. No. 9)
#1224 5'-CGCCAGGGTTTCCCAGTCACGA-3'	G	(SEQ. ID. No. 8)
C374Ap 5'-AGGTATTGGCGCCTACCCAAGTGAG-3'	T	(SEQ. ID. No. 10)
T366pd 5'-ACCCGTTCAATGAAGGTATTGGGC-3'	A	(SEQ. ID. No. 11)
V383pu 5'-AACAGGCACTACGTTCTCACTTGGTA-3'	T	(SEQ. ID. No. 12)

Figure 14

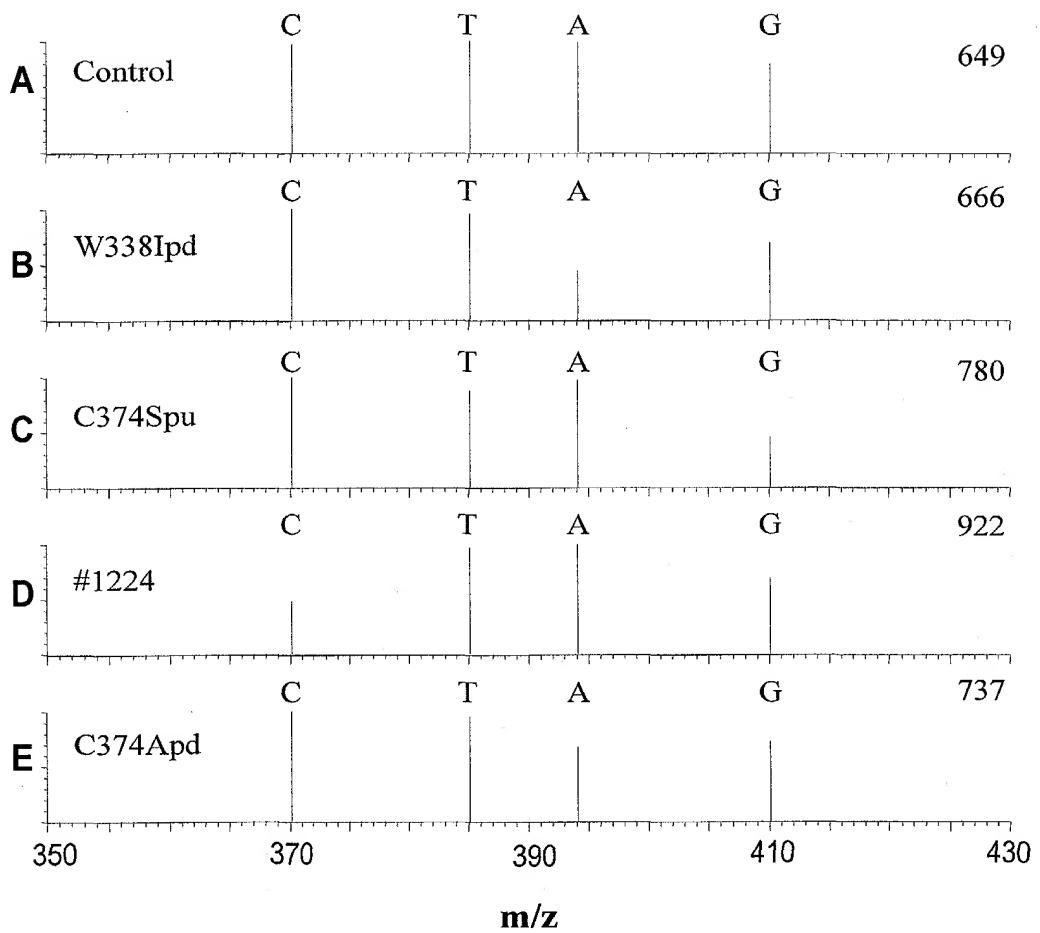


Figure 15

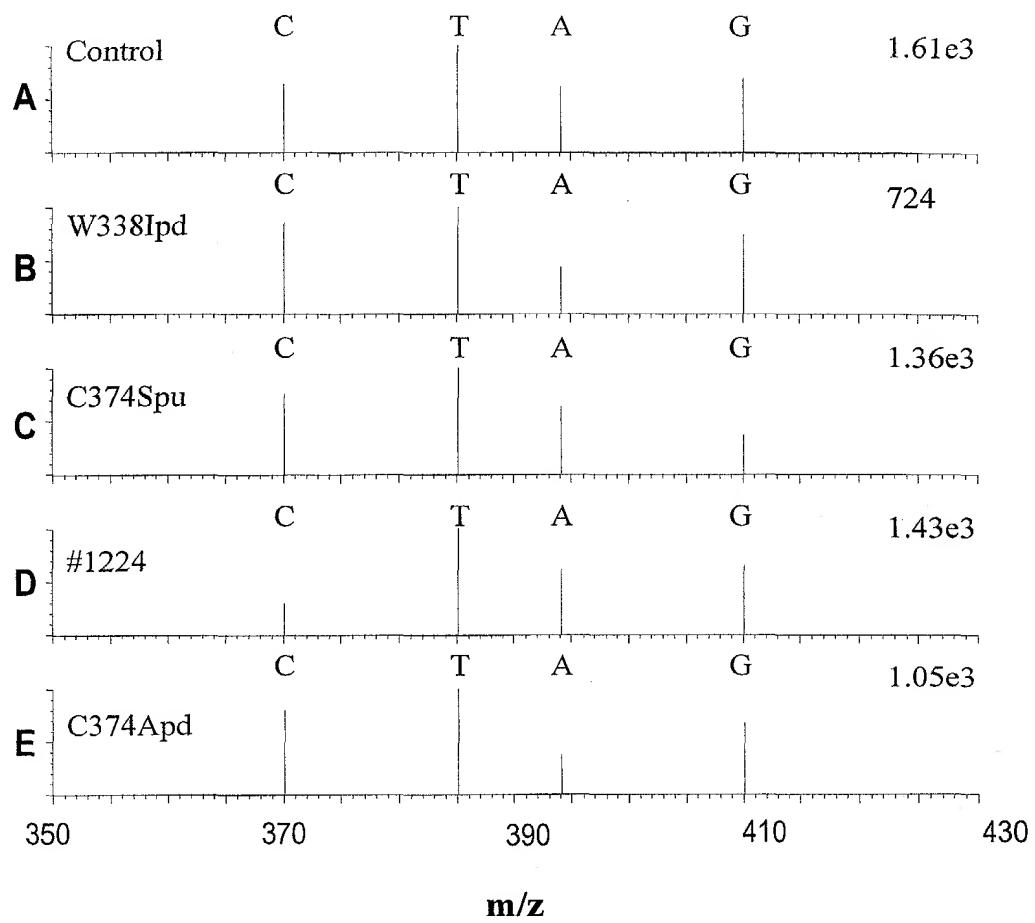


Figure 16

384 bp of partial *PheA*-C374A mutant gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 13)

5' CGGTAATCCA TGGGAAGAGA TGTTCTATCT GGATATTCAAG GCCAATCTTG AATCAGCGGA
3' GCCATTAGGT ACCCTTCTCT ACAAGATAGA CCTATAAGTC CGGTTAGAAC TTAGTCGCCT

5' AATGCAAAAA GCATTGAAAG AGTTAGGGGA AATCACCGT TCAATGAAGG TATTGGGCGC
3' TTACGTTTTT CGTAACCTTC TCAATCCCCT TTAGTGGCA AGTTACTTCC ATAACCCGAC

5' CTACCCAAGT GAGAACGTAG TGCCTGTTGA TCCAACCTGA TGAAAAGGTG CCGGATGATG
3' GATGGGTTCA CTCTTGCATC ACGGACAACT AGGTTGGACT ACTTTCCAC GGCCTACTAC

5' TGAATCATCC GGCACCTGGAT TATTACTGGC GATTGTCATT CGCCTGACGC AATAACACGC
3' ACTTAGTAGG CCGTGACCTA ATAATGACCG CTAACAGTAA GCGGACTGCG TTATTGTGCG

5' GGCTTCACT CTGAAAACGC TGTGCGTAAT CGCCGAACCA GAATTCGAGC TCGGTACCCG
3' CCGAAAGTGA GACTTTGCG ACACGCATTA GCGGCTTGGT CTTAAGCTCG AGCCATGGC

5' GGGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC
3' CCCTAGGAGA TCTCAGCTGG ACGTCCGTAC GTTCGAACCG TGACCGGCAG CAAAATGTTG

5' GTCGTGACTG GGAAAACCTT GGCG 3'
3' CAGCACTGAC CCTTTGGGA CCGC 5'
← #1224

Amplification primers:

W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3' (SEQ. ID. No. 7)
#1224 5'-CGCCAGGGTTTCCCAGTCACGA-3' (SEQ. ID. No. 8)

Polymorphism detection primers:

		Polymorphic base	
W338Ipd	5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	A	(SEQ. ID. No. 7)
#1224	5'-CGCCAGGGTTTCCCAGTCACGA-3'	C	(SEQ. ID. No. 8)
T366pd	5'-ACCCGTTCAATGAAGGTATTGGGC-3'	C	(SEQ. ID. No. 11)
V383pu	5'-AACAGGCACTACGTTCTCACTTGGTA-3'	C	(SEQ. ID. No. 12)

Figure 17

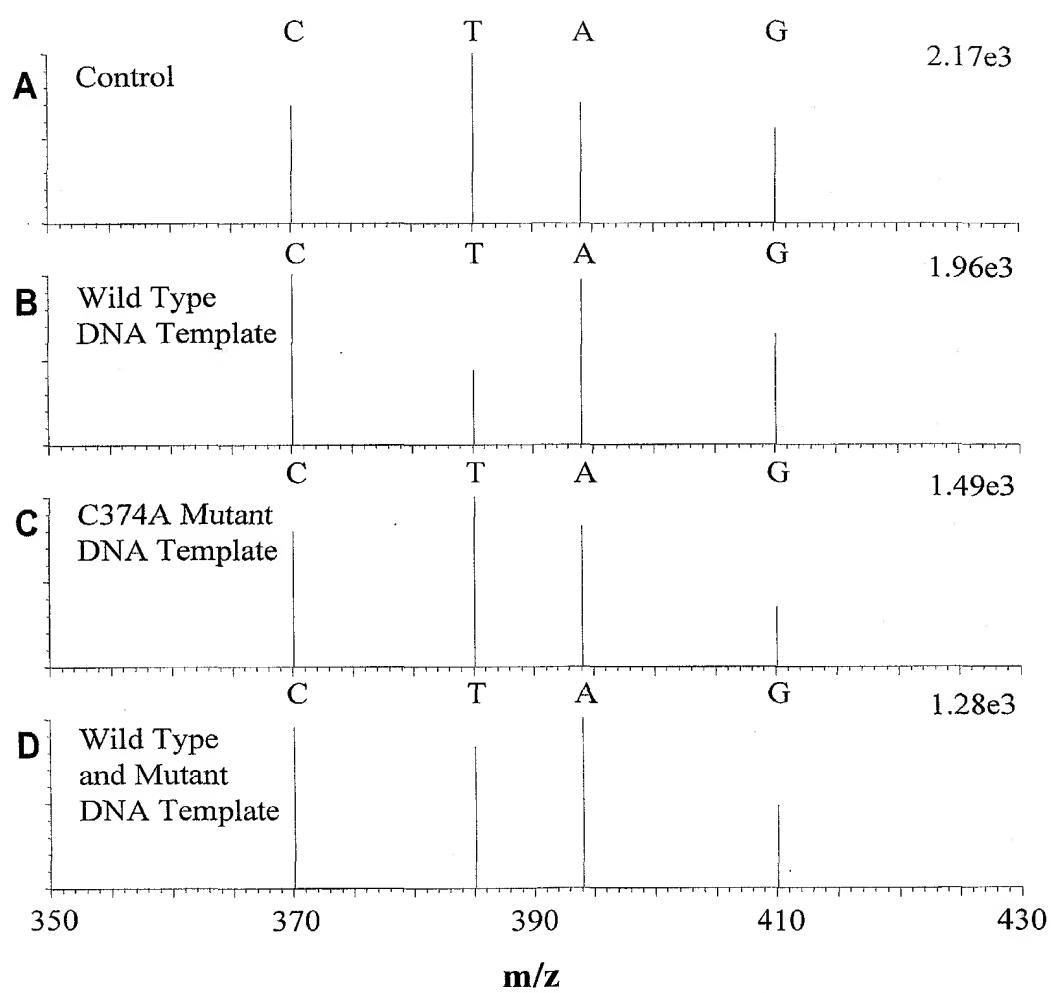


Figure 18

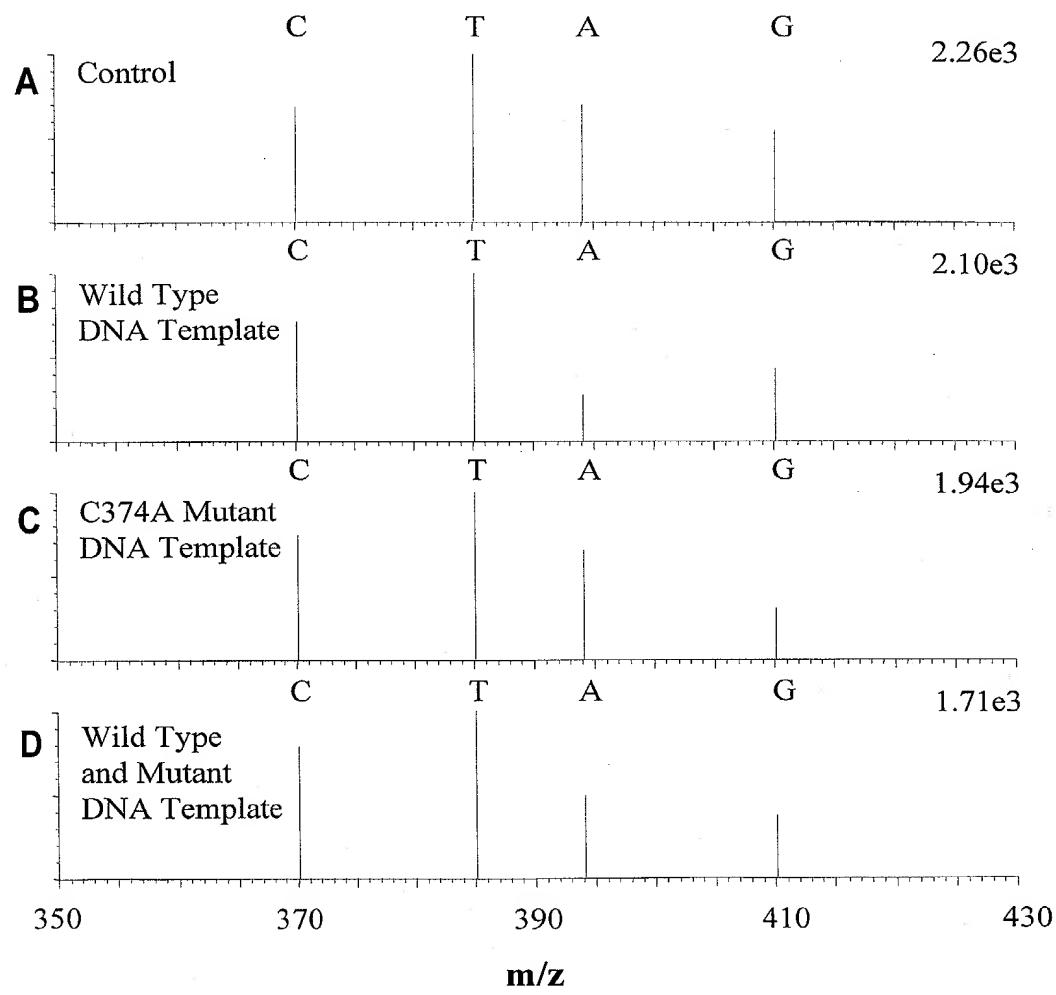


Figure 19

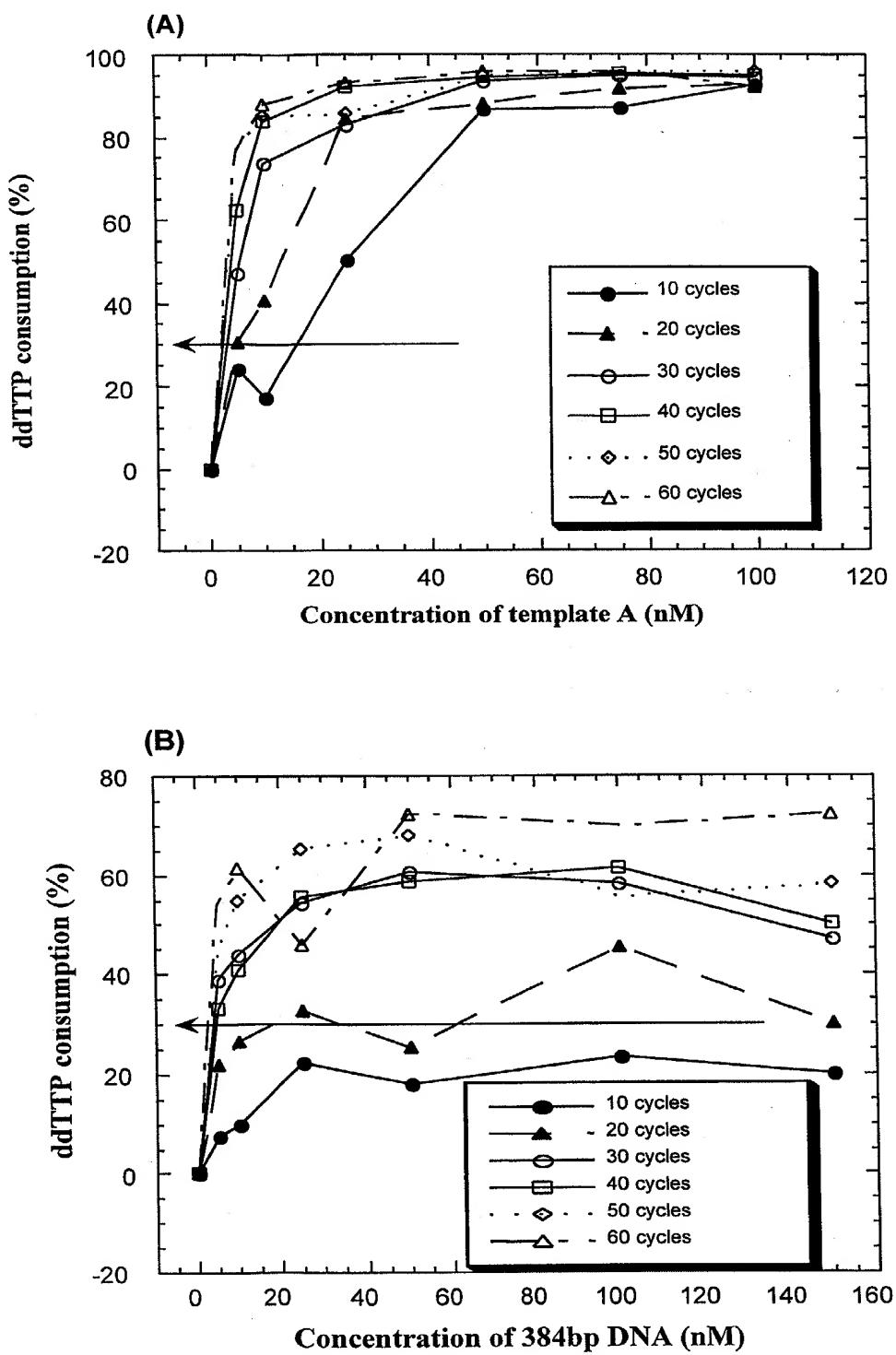


Figure 20